"Inflammaging and Platelet Hyperreactivity:- A new therapeutic target?"

Price, Joshua; Lord, Janet; Harrison, Paul

Neighborhood Watch

Inflammaging and Platelet Hyperreactivity:- A new therapeutic target?

Joshua Price, Janet M Lord, Paul Harrison. Institute of Inflammation and Ageing, College of Medical and Dental Sciences, University of Birmingham, B15 2TT, United Kingdom.

Correspondence to: Dr Paul Harrison,

p.harrison.1@bham.ac.uk,

phone -44-121-371-3251.

Inflammaging is defined as a chronic low-grade and systemic inflammation that accelerates the process of biological aging and is associated with many age-related diseases including cardiovascular disease (CVD), Rheumatoid Arthritis (RA), Myeloproliferative disease, Inflammatory Bowel disease, Alzheimer's disease, and Frailty (1). There are many contributors to this inflammatory status including adiposity, senescent cells, and aged monocytes which secrete a low level of pro-inflammatory cytokines in the absence of antigenic challenge (2). Senescent cells are metabolically active, non-proliferative, but highly pro-inflammatory cells that accumulate in tissue and organs throughout the body in association with age-related decline in the innate immune system's clearance capability (e.g. natural killer cells) (3). Aging is of course strongly associated with increased risk of CVD, the leading cause of worldwide mortality (4). Platelets play a vital role in normal haemostasis and are key players in the pathogenesis of atherothrombosis. These abundant anuclear cells not only directly mediate thrombosis but are now recognised as true inflammatory cells that can both propagate inflammatory responses and directly respond to inflammation (5). Antiplatelet drugs are therefore important for the prevention of thrombotic events in high risk patients with established CVD.

Intrinsic platelet reactivity varies between individuals and increases with age (6, 7). In older individuals, platelet hyperreactivity therefore occurs more commonly and is associated with chronic age-related CVD, comorbidities and mortality. Furthermore, underlying platelet reactivity can significantly affect responsiveness to antiplatelet drugs used to prevent thrombosis (8-10). Although the mechanisms that govern platelet reactivity in age are multifactorial (e.g. genetics, poor glucose control, dyslipidemia and oxidative stress), the precise pathways linking inflammaging to platelet function are not yet fully defined. Despite this, inflammatory cytokines such as TNF-α, IL-1β, IL-8 and IL-6 are elevated with age and associate with a suite of inflammatory conditions and CVD. Inflammatory mediators can also modify platelet function. For example, IL-6 has been implicated in altering the Megakaryocytic/Platelet axis, potentially leading to polyploidization and consequent thrombopoiesis with a shift towards a more prothrombotic phenotype and a higher mean platelet volume (MPV) (11). Furthermore platelets express GP130 which can bind to complexes of IL-6 and soluble IL-6 receptor α (sIL-6Rα) to further prime platelets via transcellular signaling and further increase their reactivity during inflammation (12). The incidence of CVD as a comorbidity in age-related diseases is also high suggesting a common pathophysiology mediated by inflammatory cytokines. Acquired platelet hyperreactivity is thus an important modifiable phenotype that forms an attractive therapeutic target for an aging population at risk of chronic diseases mediated by inflammaging.

A recent paper by Davizon-Castillo et al in *Blood* now further establishes how platelet hyperreactivity is driven by chronic inflammation (13). An excellent commentary on this article by Podrez was also in the same issue (14). This research builds upon a series of studies from some of the authors studying altered platelet function in aging. The authors now demonstrate that the pro-inflammatory cytokine TNF-α drives metabolic reprogramming of megakaryocytes (MK), platelet mitochondrial dysfunction and platelet hyperreactivity as part of normal aging in humans and mice. Furthermore, exogenous administration of TNF-α to young mice also recapitulated the aging platelet phenotype. Older mice had increased plasma levels of TNF-α and increased platelet counts but with no change in leukocyte count, in agreement with previous studies (15, 16). Upon stimulation, washed murine platelets from older mice exhibited heightened αIIbβ3 integrin expression, increased phosphatidylserine exposure and formed larger thrombi more rapidly on collagen coated slides. Interestingly it has been shown previously that TNF-α levels are much higher in the bone marrow compartment when compared to plasma in aged mice suggesting that local bone marrow MKs will be more susceptible to inflammaging (17).

There is good evidence implicating monocytes as a source of both bone marrow and systemic increases in pro-inflammatory cytokine levels, including TNF-α (18). Furthermore, both this paper and other studies have shown that monocyte derived TNF-α is implicated in the increased platelet count, reactivity and mitochondrial mass in patients with myeloproliferative disease (19, 20). It is intriguing that activated platelets also avidly interact with and bind to monocytes, further resulting in significant upregulation of the production of inflammatory cytokines (21). In a related study by some of the same authors, aging platelets have been shown to not only exhibit altered transcriptomes but to contain a 9-fold increase (compared to younger adults) in levels of granzyme A, a serine protease not previously identified in human platelets (22). This was also shown to upregulate inflammatory cytokine synthesis (e.g. IL-8 and MCP-1) in monocytes via TLR-4 and caspase 1. Although TNF-α levels were not reported in that study, it is possible that there could be significant amplification of cytokine production and platelet reactivity through this pathway in aging (23). Changes in platelet reactivity are further supported by the increase in platelet-derived products measured in plasma in healthy older participants, and further increases in the context of arterial disease including alpha granule contents and platelet-derived microparticles (24).

The increase in platelet-derived microparticles may in turn accentuate the monocyte pro-inflammatory phenotype and encourage a thrombo-inflammatory cascade effect (25).

Aging-associated transcriptional changes in MKs were measured at the single cell level. In addition to identifying temporal clusters of MKs that represent maturation status, the authors identified key pathways that were differentially regulated with age. Metabolic pathways and mitochondrial dysfunction were implicated as key age-associated changes. This may align with the observed increase of mitochondrial mass in patients with myeloproliferative disease, which is associated with chronic inflammation, with TNF-α strongly implicated. The single most highly upregulated transcript in older mouse MKs was class 1A aldehyde dehydrogenase (ALDH1A), which was also shown to be highly expressed in platelets. Metabolomic analysis also highlighted elevated pentose phosphate pathway intermediates. Increased ALDH1A and elevated pentose phosphate pathway activity is indicative of increased oxidative stress and is probably an attempted compensatory response to mitigate increased oxidative stress due to damage from free radicals and inflammation-associated damage. The increased reactivity of aged platelets may be due to the increased metabolic activity in resting aged platelets. Increased mitochondrial mass may also result in increased release of microparticle associated and free mitochondria upon platelet activation further exacerbating systemic inflammation (27). Chronic exposure to TNF-α in young mice over the course of 20 days also promoted a transition to the older MK transcriptome and recapitulated platelet hyperreactivity and the increased platelet mass observed in older mice. Strikingly the authors show that TNF-α- blockade in older mice over the course of 10 days could resolve platelet hyperreactivity and restored normal $\alpha IIb\beta_3$ expression in response to thrombin. TNF-α blockade reduced mitochondria mass but did not reduce platelet count. The TNF-α pathway was essential for the development of platelet hyperreactivity and increased mitochondrial mass as illustrated by the lack of effect of exogenous administration of TNF-α to TNF receptor deficient (p55/p75 KO) mice. Indeed bulk RNA-seq analysis confirmed that the MKs from old mice were transcriptionally homogeneous and to MKs from young mice exposed to TNF-α.

For the first time a well-established causative link between inflammaging and platelet associated hyperreactivity has been demonstrated. This opens the door to further probing TNF-α inhibition as a possible adjunct to existing preventative measures (e.g. antiplatelet drugs) to further reduce thrombotic risk and CVD. Proof of concept has been shown in RA patients receiving anti-TNF-α therapy resulting in a reduction in inflammation and platelet reactivity (28, 29). The increased incidence of CVD associated with RA also supports the importance of the thrombo-inflammatory pathways outlined by Davizon-Castillo et al. However, long-term use of TNF-α inhibitors has been associated with an adverse safety profile in RA trials (30). Interestingly, the safety profile of TNF-α inhibitors increases for ankylosing spondylitis; these patients are typically younger and receive monotherapy so may be a better cohort to establish a true safety profile (30). Nevertheless, identification of this inflammatory pathway is an exciting advance and may lead to new therapeutics. This research further emphasizes the interplay between aging, platelets and monocytes that results in inflammaging, platelet hyperreactivity and immunothrombosis.

Conflict of Interest

None of the authors has any conflict of interest

Author Contribution

Joshua Price, Janet Lord and Paul Harrison prepared the manuscript

References

1. Franceschi C, Garagnani P, Parini P, Giuliani, Santoro A. Inflammaging: a new immune–metabolic viewpoint for age-related diseases. Nature Reviews Endocrinology. 2018;14:576-90.
2. Pinti M, Appay V, Campisi J, Frasca D, Fulop T, Sauce D, et al. Aging of the immune system: Focus on inflammation and vaccination. Eur J Immunol. 2016;46(10):2286-301.
3. Prata L, Ovsyannikova IG, Tchkonia T, Kirkland JL. Senescent cell clearance by the immune system: Emerging therapeutic opportunities. Semin Immunol. 2019:101275.
4. Roth GA, Johnson C, Abajobir A, Abd-Allah F, Abera SF, Abyu G, et al. Global, Regional, and National Burden of Cardiovascular Diseases for 10 Causes, 1990 to 2015. J Am Coll Cardiol. 2017;70(1):1-25.
5. Morrell CN, Aggrey AA, Chapman LM, Modjeski KL. Emerging roles for platelets as immune and inflammatory cells. Blood. 2014;123(18):2759-67.
6. Alfredsson J, Swahn E, Gustafsson KM, Janzon M, Jonasson L, Logander E, et al. Individual long-term variation of platelet reactivity in patients with dual antiplatelet therapy after myocardial infarction. Platelets. 2019;30(5):572-8.
7. Troussard X, Vol S, Cornet E, Bardet V, Couaillac JP, Fossat C, et al. Full blood count normal reference values for adults in France. J Clin Pathol. 2014;67(4):341-4.
8. Kumar A, Kao J. Platelet resistance to antiplatelet drugs. Recent Pat Cardiovasc Drug Discov. 2009;4(2):98-108.
9. Jagroop IA, Matsagas MI, Geroulakos G, Mikhailidis DP. The effect of clopidogrel, aspirin and both antiplatelet drugs on platelet function in patients with peripheral arterial disease. Platelets. 2004;15(2):117-25.
10. Yee DL, Sun CW, Bergeron AL, Dong JF, Bray PF. Aggregometry detects platelet hyperreactivity in healthy individuals. Blood. 2005;106(8):2723-9.

11. Burstein SA, Peng J, Friese P, Wolf RF, Harrison P, Downs T, et al. Cytokine-induced alteration of platelet and hemostatic function. Stem Cells. 1996;14 Suppl 1:154-62.
12. Houck KL, Yuan H, Tian Y, Solomon M, Cramer D, Liu K, et al. Physical proximity and functional cooperation of glycoprotein 130 and glycoprotein VI in platelet membrane lipid rafts. J Thromb Haemost. 2019;17(9):1500-10.
13. Davizon-Castillo P, McMahon B, Aguila S, Bark D, Ashworth K, Allawzi A, et al. TNF-α–driven inflammation and mitochondrial dysfunction define the platelet hyperreactivity of aging. Blood. 2019;134(9):727-40.
14. Podrez E. Platelet hyperreactivity: a new twist in old mice. Blood. 2019;134(9):723-4.
15. Culmer D, Diaz J, Hawley A, Jackson T, Shuster K, Sigler R, et al. Circulating and vein wall P-selectin promote venous thrombogenesis during aging in a rodent model. Thromb Res. 2013;131(1):42-8.
16. Dayal S, Wilson K, Motto D, Jr MF, Chauhan A, Lentz S. Hydrogen Peroxide Promotes Aging-Related Platelet Hyperactivation and Thrombosis. Circulation. 2013;127(12):1308-16.
17. Puchta A, Naidoo A, Verschoor CP, Loukov D, Thevaranjan N, Mandur TS, et al. TNF Drives Monocyte Dysfunction with Age and Results in Impaired Anti-pneumococcal Immunity. PLoS Pathog. 2016;12(1):e1005368.
18. Parameswaran N, Patial S. Tumor necrosis factor-alpha signaling in macrophages. Crit Rev Eukaryot Gene Expr. 2010;20(2):87-103.
19. Taylor DD, Senhauser DA, Cavazos F. Thrombocytopathy associated with nonleukemic megakaryocytic myelosis. Functional and fine structure observations of the abnormal platelets. Am J Clin Pathol. 1968;49(5):662-70.
20. Hattori A, Koike K, Ito S, Matsuoka M. Static and functional morphology of the pathological platelets in primary myelofibrosis and myeloproliferative syndrome. Ser Haematol. 1975;8(1):126-50.
21. Stephen J, Emerson B, Fox KA, Dransfield I. The uncoupling of monocyte-platelet interactions from the induction of proinflammatory signaling in monocytes. J Immunol. 2013;191(11):5677-83.
22. Campbell RA, Franks Z, Bhatnagar A, Rowley JW, Manne BK, Supiano MA, et al. Granzyme A in Human Platelets Regulates the Synthesis of Proinflammatory Cytokines by Monocytes in Aging. J Immunol. 2018;200(1):295-304.
23. Montenont E, Rondina MT, Campbell RA. Altered functions of platelets during aging. Curr Opin Hematol. 2019;26(5):336-42.
24. Lebas H, Yahiaoui K, Martos R, Boulaftali Y. Platelets Are at the Nexus of Vascular Diseases. Front Cardiovasc Med. 2019;6(132).
25. Chimen M, Evryviadou A, Box C, Harrison M, Hazeldine J, Dib L, et al. Appropriation of GPIbα from platelet-derived extracellular vesicles supports monocyte recruitment in systemic inflammation. Haematologica. 2019.
26. Grabowska W, Sikora E, Bielak-Zmijewska A. Sirtuins, a promising target in slowing down the ageing process. Biogerontology. 2017;18(4):447-76.
27. Boudreau LH, Duchez AC, Cloutier N, Soulet D, Martin N, Bollinger J, et al. Platelets release mitochondria serving as substrate for bactericidal group IIA-secreted phospholipase A2 to promote inflammation. Blood. 2014;124(14):2173-83.
28. Gasparyan AY, Kitas GD. Platelets in rheumatoid arthritis: exploring the anti-inflammatory and antithrombotic potential of TNF inhibitors. Ann Rheum Dis. 2016;75(8):1426-7.
29. Mac Mullan PA, Peace AJ, Madigan AM, Tedesco AF, Kenny D, McCarthy GM. Platelet hyper-reactivity in active inflammatory arthritis is unique to the adenosine

diphosphate pathway: a novel finding and potential therapeutic target. Rheumatology (Oxford). 2010;49(2):240-5.
30. Wroński J, Fiedor P. The Safety Profile of Tumor Necrosis Factor Inhibitors in Ankylosing Spondylitis: Are TNF Inhibitors Safer Than We Thought? J Clin Pharmacol. 2019;59(4):445-62.